

Hyperspectral vision for early detection of yeast presence in organic medium (agar)

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Abstract

Predictive microbiology develops mathematical models that describe microbial behavior in food. Its efficiency is limited in foods showing some degree of structure, which are the majority, because these models are based on experimental data obtained in liquid foods, much more homogeneous than solids or semi-solids. In this paper we evaluate the hyperspectral analysis technique as a technology for early detection of yeasts in solid food by principal component analysis and External Parameter Orthogonalisation EPO, this last procedure employed for the elimination of the agar influence on the spectra of yeast colonies. Both analysis are shown and discussed. Hyperspectral images (400-1000 nm) were acquired from Petri plates inoculated with *Zygosaccharomyces rouxii* Bch. at different times along the days of development of the colonies. The score images computed from the application of the principal component to the hyperspectral images show that the first component discriminates between the agar and the colonies, while the second shows a greater variability in the colonies. This noninvasive tool characterizes the state of development, number and size of colonies, which would provide data to develop reliable models to predict the risk of deterioration and shelf life of foods.

Key words: artificial vision, multivariate analysis, food safety, yeast contamination

1. Introduction

Predictive microbiology, through mathematical models of microbial behavior in foods is an essential tool in improving the quality and safety of them. Yeasts are recognized agents of deterioration in foods (Deak, 2007) that are increasingly considered also in solid foods. In recent years, has increased the demand for vegetables, fruits, and generally minimally processed and ready-to-eat products. These foods have a higher risk of microbial spoilage, mainly because they have not been subjected to processes that reduce the microbial load and also tend to be confined in small packages. The acidity, the use of modified atmospheres, and other factors favor the growth of yeasts, spoilage agents becoming frequent and sometimes dominant in this type of food (Jacxsens et al., 2002; Tournas et al., 2006).

The internal structure of the food is a key factor in the behavior of the contaminating organisms (Hills et al., 2001). However, current methodologies for detecting microorganisms in food solids are destructive. Therefore, hyperspectral imaging could provide a non-

destructive technique to detect changes that occur in food due to contamination by yeasts, as they appear related to variations measured in the spectra at wavelengths in the range of visible and near infrared. This technique has been applied previously to detect contamination in vegetables by bacteria and fungi (Liu et al., 2007; Delwiche et al., 2011, Silva et al., 2011). This study was conducted with *Zygosaccharomyces rouxii* Bch, a major yeast species deteriorating food and drinks. This yeast is isolated from foods of intermediate water activity, such as concentrated syrups, fruit juices, marzipan and honey, among others. The addition of preservatives to some foods may cause its deterioration, due to the transformation of sorbate in 1-3-pentadiene by certain microbial species. This is the case of the capacity of some strains of molds and yeasts that are capable of transforming decarboxilar sorbic acid in 1-3 pentadiene, a volatile compound with an unpleasant odor of petroleum (Casas, 1999). The global aim of this research will be to establish reliable models that allow the detection of yeast in solid media, predicting the risk of deterioration and shelf life of foods, by means of hyperspectral imaging techniques. The present paper shows preliminary results of hyperspectral images for the detection of the presence of yeast in an organic medium (agar).

2. Materials and methods

2.1 Microbial growth conditions

Zygosaccharomyces rouxii Bch, is a yeast strain isolated from a food deteriorated in our laboratory and maintained routinely in pipes YMA (Yeast Morphology Agar) at 28 ° C. This strain was inoculated, from a fresh culture for 48 h in 250 ml flasks containing 100 ml capacity of YMB medium (Yeast Morphology Broth). After incubation with stirring for 18 h at 28 ° C, optical density was measured at 620 nm and determined the number of colony forming units / ml by interpolation from a standard curve. Proceeded to the embodiment of decimal dilutions in saline (9 g / l NaCl, Panreac Chemistry, Barcelona, Spain) and the inoculation of 50 ml drops of the appropriate dilution, which was expected to be obtained one single CFU, regions of 4 cm² labeled petri dishes (85 mm Ø) with 15 ml of YMA (10g / l glucose), in order to take hyperspectral imaging of the region before the growth was visible (Figure 1).

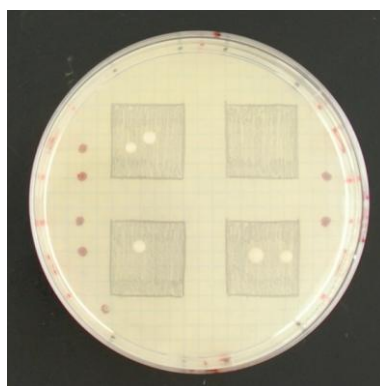


FIGURE 1: Disposition of the Petri dishes to acquire hyperspectral images. The inoculation was performed in the corresponding area of the squares.

2.2. Hyperspectral images acquisition and analysis

Three inoculated Petri dishes were supervised for a six-day period. Hyperspectral images were acquired over time, just after inoculation and after one, two, three and six days. The hyperspectral vision system consisted of a linear CCD detector push-broom coupled to a spectrograph Headwall Photonics VNIR (400 to 1000 nm, 3 nm spectral resolution, with acquisition time of 275 ms).

Several multivariate analyses were applied to the hyperspectral images in order to discuss which procedure suits best for the early determination of the presence of yeast

contamination. Once the hyperspectral images were acquired, they were preprocessed and processed.

A calibration set was considered with representative spectra of the medium agar, and of yeast belonging to different test dates (N=418 total of spectra from which n=264 spectra of agar 132 belonging to the first and 132 to the second date, m=154 spectra of yeast: 103 belonging to first date, 51 to second date). These spectra were manually selected from agar as well as from yeast visually identified areas.

Since yeast colonies grow on agar their spectral responses could be influenced by the agar. Thus, the reflectance spectra and hyperspectral images acquired from the colonies of yeast could be influenced by the properties (composition, physical properties, color) of the agar. Therefore, external parameter orthogonalisation (EPO) developed by Roger (2003) was applied to the calibration set and to the hyperspectral images for removing the effect of agar on the spectra of yeast. EPO is deeply explained by Roger (2003). Briefly, it is a method that estimates a parasitic subspace by computing a principal component analysis on a set of representative spectra of agar (X_{agar}) in different stages. Afterwards this agar subspace is removed from the spectra of yeast X (m spectra, p wavelengths) by orthogonal projection of the yeast on the agar, obtaining X* (m spectra, p wavelengths): matrix of spectra of yeast after removing the agar influence. The orthogonalisation procedure was applied to eliminate the first component of the agar subspace, namely 'epo 1' in the present research; the first and the second 'epo 2', the first, the second and the third 'epo 3' respectively. Further, these preprocessed spectra of yeast were again put together with the spectra of agar, reconstituting new calibration set of spectra. Then, four calibration set of spectra were considered each composed by N=418 spectra (264 of agar and 154 of yeast). These four calibration sets included respectively the raw, epo 1, epo 2 and epo 3 spectra of yeast.

Four Principal Component Analysis have been applied on these four calibration sets, obtaining their corresponding loadings and scores. ANOVA was computed on these scores aiming to show which method discriminates most between agar and yeast.

Finally, images of scores were obtained by the projection of the hyperspectral images on the resulting loadings obtained from the calibration sets of spectra. In the case of EPO consideration, the hyperspectral images were preprocessed before their score images computation.

3. Results

Firstly the analysis was applied without EPO preprocess. The spectra belonging to the calibration set were employed for the computation of the principal component analysis and after they were projected on the plane formed by the first two principal components. (Fig. 2, left). Scores resulting from such projection showed clear differences between agar and yeast. First component completely separates yeast and agar scores, while the second component distinguishes differences in agar, probably due to hydration stage. Scores of yeast appear more disperse than agar, probably due to the fact that the different cells of the colonies present different stage of development or activity. Also it could be due to the influence of agar response on the spectra of different regions of the colonies.

PC score images were obtained from the projection of hyperspectral images on the first principal component (Fig. 2, right); the size and surface of colonies increases with time and quantification is computed accordingly. Hence these score images could be used to quantify the size and surface of the colonies at different dates (Figure 2, right) with the advantage of improving contrast and colonies segmentation. This analysis has also been applied by Wang et al., (2012) to detect infected areas in onion through hyperspectral images.

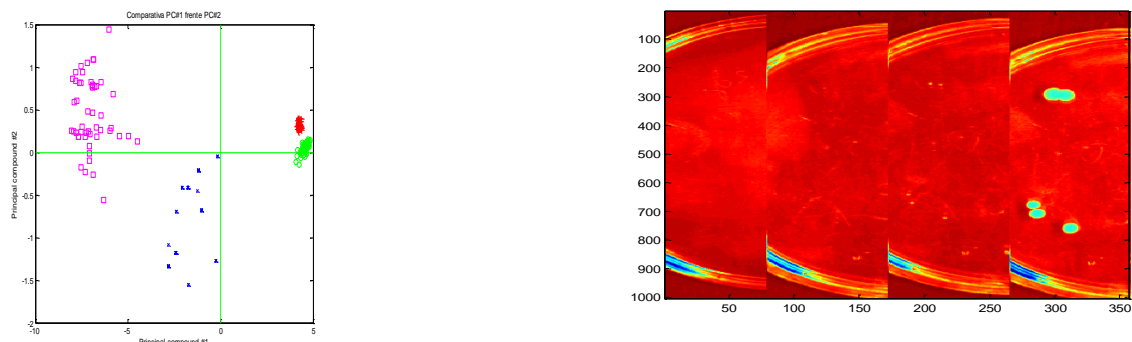


FIGURE 2: Left, PC1 and PC2 scores of the agar (red +, green o) and yeast (pink □, blue x) (n=418 spectra). Right, score images obtained from the projection of the hyperspectral onto PC1 (from left to right, just inoculated, after one, two, three and six days).

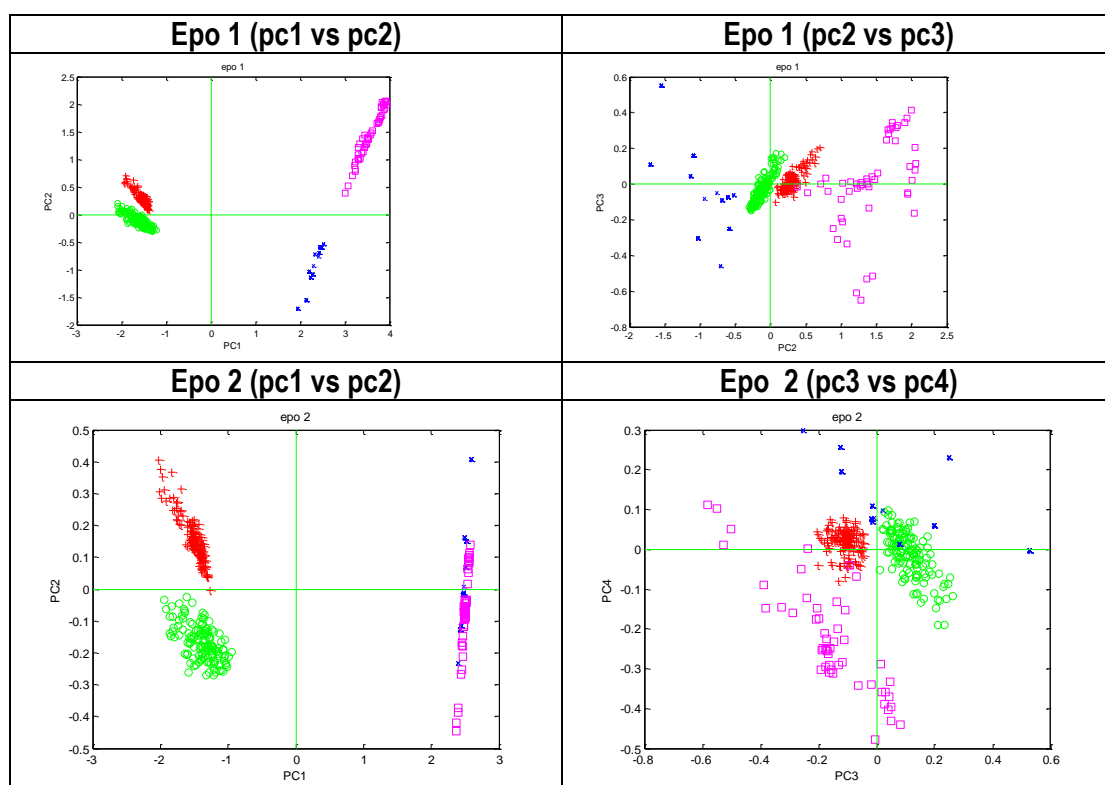


FIGURE 3: Scatter plot of pca scores of the calibration set of spectra (n=418) corrected by EPO: first line epo 1 scores, second line epo 2 scores. The scores of the agar (red +, green o) and yeast (pink □, blue x).

Figure 3 shows the PC-scores corresponding to pca on the preprocessed spectra. For both types of preprocessed spectra: epo 1 and 2, the first principal component discriminates between agar and yeast, with lower variability for the scores of yeast than for agar (as expected after having performed an orthogonalisation). As for epo 1, the second component presented higher variability for yeast than for the agar, which could indicate that it is able to distinguish between several stages of yeast, while agar discrimination is highest within the scores of the third component.

TABLE 1: ANOVA results comparing pc1 scores of agar n= 264 and yeast n= 154 obtained from pca applied to the preprocessed calibration sets.

Calibration set	F (Fisher)	P level
Raw data	2472	**
Epo 1 (epo eliminating one component)	11603	**0
Epo 2 (epo eliminating two component)	54216	**0
Epo 3 (epo eliminating three component)	51135	**0

All four pca procedures show that the first component retains the variability between agar and yeast. Aiming to discuss which procedure is the most discriminant, four ANOVAS were applied on the scores. The highest F-Fisher was found for epo 2, when the first two main direction of agar were removed from spectra of yeast. A slight decrease was observed for epo 3, comparing it with epo 2. Therefore it shows that there is no need to remove the first three main directions of agar and only two apparently are enough.

When searching for the goal distinguishing between yeast stages, the most discriminant direction corresponded to pc2 in epo 1, and to pc3 in epo 2. Therefore these loadings should be applied for the projection of the hyperspectral images in order to enhance discrimination contrast.

Figure 4 shows the resulting score image of epo 2 projected onto pc1. It shows globally similar pattern that score image without epo (figure 2, right). However it will needed to develop further analysis to define precisely the differences.

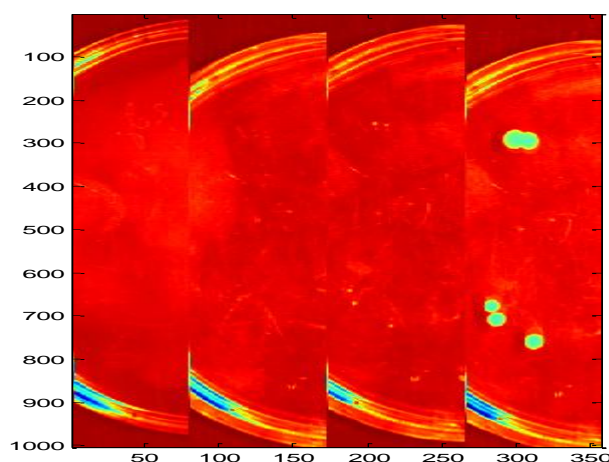


Figure 4. Score images obtained from the projection of the preprocessed epo 2 hyperspectral onto PC1 (from left to right, just inoculated, after one, two, three and six days).

Conclusions

The images of the scores of agar and yeast corresponding to the Principal Component Analysis, show the potential of gathering spectroscopy and vision as tools for detection of yeast. Further experiments are needed for validation, where the differences in the stage of development of the colonies could be identified, and more detailed images could be analyzed. In addition, multispectral vision (few wavelengths) could be proposed. Furthermore, one can propose a wavelength selection related to the development of the colonies, with the aim of proposing a future multispectral vision system as recently proposed for other authors.

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